

WHAT IS CLAIMED IS:

1. A method of detecting a mismatch between two substantially complementary DNA sequences of interest, the method comprising:

annealing a first strand comprising a gene encoding a detectable marker and an origin of replication active in a bacterial host cell, wherein said first strand is characterized by the absence of methyl adenine; and a substantially complementary second strand, wherein said gene encoding said detectable marker further comprises an inactivating insertion, deletion or substitution of at least about 5 nt, and characterized by the presence of methyl adenine at GATC sites;

ligating said first strand to a first DNA sequence of interest of from about 20 to  $10^4$  nucleotides in length;

ligating said substantially complementary second strand to a second DNA sequence of interest substantially complementary to said first DNA sequence, and suspected of having at least one mismatch of from 1 to 4 contiguous nucleotides in length;

circularizing said ligated first and second strands to provide a circular double stranded DNA molecule;

transforming a bacterial host having an active methyl mismatch repair system with said circular double stranded DNA molecule;

detecting the presence of bacterial transformants not expressing said detectable marker;

wherein the presence of transformants not expressing said detectable marker is indicative of a mismatch between said first DNA sequence of interest and said second DNA sequence of interest.

2. A method according to Claim 1, further comprising isolating and growing said bacterial transformants.

3. A method according to Claim 1, wherein said first strand and said substantially complementary second strand further comprise a selectable marker, and a polylinker having multiple sites for restriction endonucleases.

5 4. A method according to Claim 1, wherein said first DNA sequence of interest is a polymerase chain reaction product.

5. A method according to Claim 1, wherein said first DNA sequence of interest is a cDNA.

10 6. A method according to Claim 4, wherein said first DNA sequence of interest is a restriction fragment.

15 7. A method according to Claim 1, wherein said second DNA sequence of interest is a polymerase chain reaction product.

8. A method according to Claim 1, wherein said second DNA sequence of interest is a cDNA.

20 9. A method according to Claim 1, wherein said second DNA sequence of interest is a restriction fragment.

10. A method according to Claim 1, wherein said ligating said first strand to a first DNA sequence of interest is performed prior to said annealing step.

25 11. A method according to Claim 1, wherein said ligating said first strand to a first DNA sequence of interest is performed after said annealing step.

12. A method according to Claim 1, wherein said ligating said substantially complementary second strand to a second DNA sequence of interest substantially complementary to said first DNA sequence is performed prior to said annealing step.

5 13. A method according to Claim 1, wherein said ligating said substantially complementary second strand to a second DNA sequence of interest substantially complementary to said first DNA sequence is performed after said annealing step.

10 14. The method of Claim 1, wherein said detectable marker is a site specific recombinase.

15 15. The method of Claim 14, wherein said site specific recombinase is cre recombinase.

16 16. The method of Claim 15, wherein the presence of active cre recombinase is detected by the absence or presence of recombination between two lox sites, wherein directly detected marker is present between said two lox sites.

20 17. The method of Claim 16, wherein said directly detected marker provides for antibiotic resistance or antibiotic sensitivity.

18. A method of detecting a mismatch between two substantially complementary DNA sequences of interest, the method comprising:

annealing in a hybridization reaction:

25 a vector comprising a gene encoding a detectable marker, an origin of replication active in a bacterial host cell, and a sequence of interest, wherein said vector lacks methyl adenine; and

a substantially complementary second vector, wherein said gene encoding said detectable marker further comprises an inactivating insertion, deletion or

substitution of at least 5 nt, wherein said vector comprises methyl adenine at GATC sites; and

a test sequence substantially complementary to said sequence of interest, and suspected of having at least one mismatch of from 1 to 4 contiguous nucleotides in length;

ligating the product of said annealing step;

transforming a bacterial host having an active methyl mismatch repair system with the product of said ligating step;

determining the presence of bacterial transformants expressing or not expressing said detectable marker;

wherein the presence of transformants not expressing said detectable marker is indicative of a mismatch between said sequence of interest and said test sequence.

19. The method of Claim 18, wherein said annealing step comprises a plurality of said vectors comprising a sequence of interest, and a plurality of test sequences.

20. The method of Claim 18, further comprising isolating and growing said bacterial transformants.

21. The method of Claim 18, wherein said test sequence is an amplification product.

22. The method of Claim 18, wherein said detectable marker is a site-specific recombinase.

23. The method of Claim 22, wherein said site specific recombinase is cre recombinase.

24. The method of Claim 23, wherein the presence of active cre recombinase is detected by the absence or presence of recombination between two lox sites, wherein directly detected marker is present between said two lox sites.

5 25. The method of Claim 18, wherein said directly detected marker provides for antibiotic resistance or antibiotic sensitivity.

26. A kit for identifying the presence of a mismatch between two substantially complementary DNA sequences, the kit comprising:

10 a first DNA vector comprising a gene encoding a detectable marker, an origin of replication active in a bacterial host cell, and a sequence(s) of interest, wherein said vector lacks methyl adenine; and

a substantially complementary second vector, wherein said gene encoding said detectable marker further comprises an inactivating insertion, deletion or substitution of  
15 at least 5 nt, wherein said vector comprises methyl adenine at GATC sites